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The impact of ethanol blend releases to the aesthetic quality of groundwater and the indigenous microbial community

by

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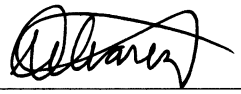

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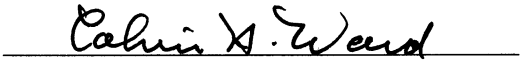

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ABSTRACT

The impact of ethanol blend releases to the aesthetic quality of groundwater and the indigenous microbial community

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A pilot-scale aquifer system was used to evaluate groundwater quality impacts from a continuous release of an aqueous 10% v:v ethanol solution mixed with benzene and toluene (50 mg/L each). The geochemical footprints (methane, volatile fatty acids (VFAs), pH, oxidation reduction potential (ORP), dissolved oxygen (DO) and temperature) were monitored over 11 months. The accumulation of VFAs (particularly butyric acid) during the summer exceeded the secondary maximum contaminant level value for odor, which represents a previously unreported aesthetic impact. Temperature variations (3.9 to 30.0 °C) significantly affected microbial activities, and a strong correlation was observed between groundwater temperature and CH_4/VFAs generation ($p < 0.05$). Quantitative real-time PCR (qPCR) analysis showed that ethanol blend increased the microbial population and enriched the fermentative groups for acetogenesis and methanogenesis.

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INTRODUCTION

Ethanol is an important gasoline additive and renewable fuel. Annual ethanol production in the US has increased from 2,130 million gallons in 2002 to 10,600 million gallons in 2009, with an average growth rate of 25.8% per year (Renewable Energy Associations 2010). The rapidly growing reliance on ethanol blend fuels has increased the potential for groundwater contamination when ethanol reaches aquifer systems during production, transportation and storage. Thus, it is important to investigate the potential environmental impacts of such releases.

Previous research has studied the migration characteristics of ethanol in the subsurface (Dakhel et al. 2003, Corseuil, Kaipper and Fernandes 2004, Stafford et al. 2009, McDowell and Powers 2003, Capiro et al. 2007, McDowell, Buscheck and Powers 2003), its impact on indigenous microorganisms (Feris et al. 2008, Capiro et al. 2008, Nelson, Lapara and Novak 2010) and its influence on the concentration and persistence of petroleum hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX) (Beller et al. 2008, Corseuil et al. 1998, Ruiz-Aguilar, O'Reilly and Alvarez 2003, Mackay et al. 2006, Lovanh, Hunt and Alvarez 2002). However, less attention has been directed towards the impact of ethanol intermediate biodegradation products on potential groundwater quality, and how these impacts may change with seasonal variations in temperature.

Ethanol biodegradation rapidly consumes oxygen and other electron acceptors creating an anaerobic environment. Under these anaerobic conditions, ethanol can be

fermented to volatile fatty acids (VFAs) such as acetic, propionic, butyric, and isobutyric acids, which can be further syntrophically transformed to hydrogen (H_2) and methane (CH_4) (Powers et al. 2001). The intermediate degradation products are ultimately mineralized (to H_2O and CO_2) under oxidizing conditions. Transient presence of VFAs, however, may cause aesthetic impacts to potable groundwater due to their odor and taste. Malodor is one of the main reasons for consumers to complaint about their drinking water quality. Even though many odorous compounds are not toxic to the human body, they affect the public's perception of the safety of drinking water. In the United States, the Environmental Protection Agency (US EPA) includes odor as one of fifteen contaminants in National Secondary Drinking Water Regulations. Furthermore, CH_4 could accumulate in shallow aquifers and subsurface soils and pose hazards at sites with subsurface confined spaces and conditions conducive to ignition (Nelson et al. 2010; Freitas et al. 2010). Temperature is an important factor that affects indigenous microbial activities (Alvarez and Illman 2005). Higher temperature can enhance enzyme activity and accelerate biodegradation rate. Low temperature may reduce the permeability of cell membranes which may hinder nutrient uptake. The temperature of the upper 10 m zone in the subsurface may vary seasonally (Lee et al. 1988). Therefore, when assessing an aquifer's capacity for natural attenuation of ethanol blends releases and characterizing impacts from by-products of ethanol biodegradation, the variations in groundwater temperature with seasonal changes should be considered.

Microorganisms play a key role in the transformation of ethanol blend fuels when they are released into the subsurface environment. The introduction of ethanol into the carbon-limited aquifer system can remarkably shift subsurface redox conditions and change the structure and function of indigenous microbial communities. As a liable compound, ethanol represents a good energy and carbon source that may stimulate the growth many kinds of microorganisms (Powers et al. 2001). However, high concentrations of ethanol are toxic to microorganisms and very few species can grow at ethanol concentrations higher than 100,000 mg/L (Ingram and Buttke 1984). The presence of ethanol may also change the function of microbial communities. The preferential biodegradation of ethanol will accelerate the consumption of electron acceptor and nutrients and hinder the biodegradation of BTEX (Corseuil et al. 1998, Lovanh et al. 2002, Lovanh and Alvarez 2004, Mackay et al. 2006, Feris et al. 2008). Although the effect of ethanol on microorganisms that degrades BTEX has been previously investigated, the impact of ethanol to the composition of microbial communities, particularly for the ethanol fermentative degradation groups, has received limited attention.

OBHECTIVES AND HYPOTHESES

A pilot-scale aquifer system was used to assess the impacts of a continuous release of a simulated fuel ethanol blend (10% v:v ethanol, 50mg/L benzene and 50mg/L toluene) on groundwater quality and indigenous microbial community. Specific objectives were to:

1. Determine how seasonal variations in groundwater temperatures affect microbial processes that could result in adverse secondary impacts; specifically methanogenesis (which could result in an explosion risk) and VFAs generation (which could generate odor).

Hypothesis: In summer, high temperatures can accelerate microbial activities. Thus, stronger methanogensis and VFAs generation would be expected. The opposite would be true for winter. A correlation between groundwater temperatures and methane/VFAs concentrations in the groundwater should exist.

2. Quantify the accumulation of VFAs and their seasonal variation within the context of potential aesthetic impacts to groundwater quality, and assess correlations with temperature and other microbial processes

Hypothesis: VFAs have malodor. The accumulation of VFAs in groundwater may impose aesthetic impact to water quality. The level of the aesthetic impact would vary seasonally due to the effects of temperature on VFAs generations.

3. Assess the microbial response to ethanol blend in terms of changes in the composition of the indigenous microbial community and the relative abundance of specific genotypes of interest to processes affecting bioremediation and groundwater quality.

Hypothesis: Ethanol blends could stimulate microbial growth and change the composition of indigenous microbial community. In particular, ethanol may

impose a selective pressure and enrich the genotype related to the fermentative degradation of ethanol blends.

This thesis is a subproject of a large collaborative project with Prof.William Rixey in the University of Houston. The data about benzene, toluene and ethanol were collected by them. In this thesis I just shows the data obtained by me.

MATERIALS AND METHODS

Pilot scale aquifer system

An 8 m3 (3.7 m×1.8 m ×1.2 m) pilot-scale continuous-flow tank packed with fine grain sand was used in this study. The tank was covered by a canopy to avoid confounding effects from rain water and was open to the atmosphere. Details on the tank construction, gravity-fed hydraulics, media, and packing methods can be found in Stafford (2007). A plan view of the tank is shown in Figure 1 and appendix II contain four pictures of the tank system. Two parallel channels separated by an acrylic barrier were equipped with independent inlet and outlet lines and instrumented with sampling ports and wells to monitor groundwater. Tap water was injected from the inlet of each channel to obtain a water table elevation of 0.75 m from the bottom of the tank. The vadose zone was 0.35 m high and the total aquifer thickness was 1.1 m. Inlet water characteristics can be found in Table 1. In Channel 1, a municipal water amended with 10 % (v/v) ethanol, 50 mg/L benzene, 50 mg/L toluene (E/B/T) and 24,000 mg/L sodium bromide (NaBr) was injected at a depth of 22.5 cm below the water table at a

rate of 0.4 L/d. The NaBr was added as a conservative tracer, and to maintain a solution density to reach a neutral buoyancy condition with the flowing groundwater. Although high salt concentrations can be inhibitory to bacteria due to osmotic stress, the added bromide salt was diluted by the tank flow to less than 5,000 mg/L, which is within the typical tolerance range of soil bacteria (Atlas and Bartha 1993). The density of the ethanol/NaBr solution injected relative to water, was measured as 1.002 at 20 °C. Channel 2 served as a control with the same injection depth and injection rate of water mixture containing 50 mg/L benzene, 50 mg/L toluene (B/T) and 24,000 mg/L NaBr with an estimated density relative to water of 1.019 at 20 oC. The monitoring network was designed to delineate the developed solute (i.e., B/T and ethanol) plumes and characterize solute degradation and accumulation of CH4 and VFAs. All sampling ports (sample ports were steel tubes screened on the bottom outlet) were at the same depth as the E/B/T mixture injection point. Vertical sampling in Channel 2 was conducted at various depths given the possibility of some downward migration.

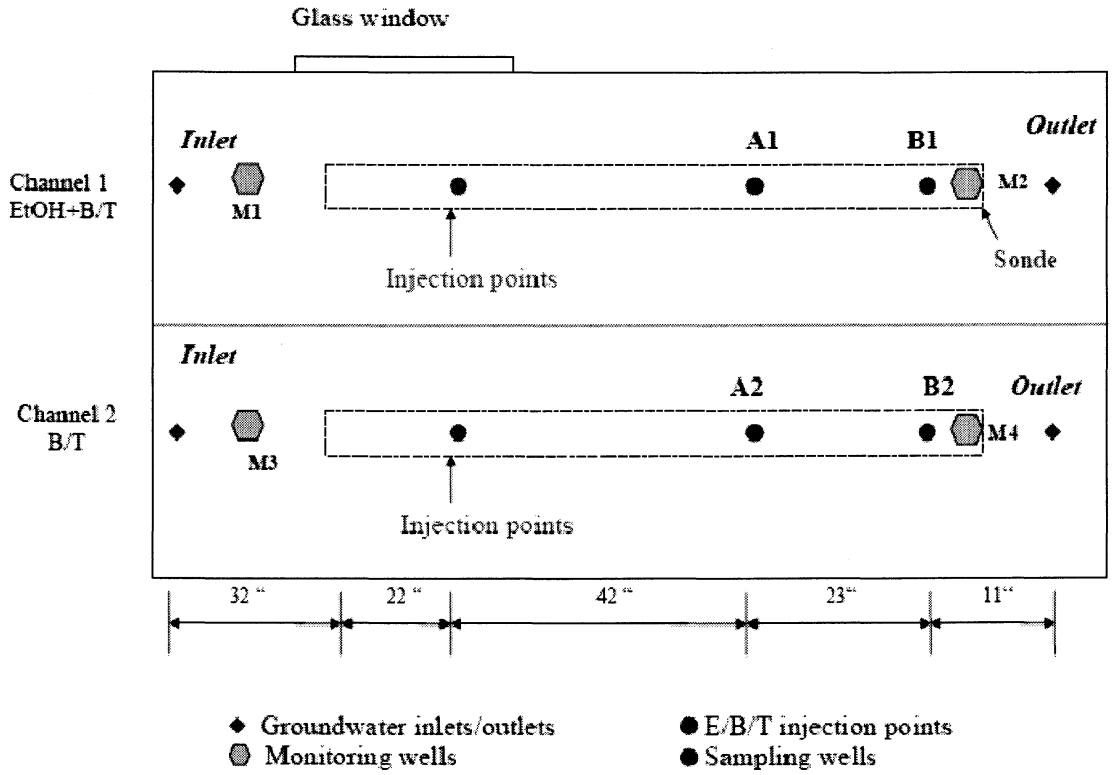

Figure 1. Plan view of the experimental release system. Both injection ports and sampling ports were located at 22.5 cm below the water table.

Methane and VFAs analysis

Aqueous samples (A1, B1 for Channel 1 and A2, B2 for Channel 2) were collected every ten days from August 7th, 2009 to June 9th, 2010 and analyzed for CH_4 and VFAs.

For CH_4 analysis, aqueous samples (50 ml) were injected into glass serum bottles (125 ml) capped with a Teflon-lined septa and aluminum crimps. Bottles were shaken on an Orbit 300 Multipurpose Vortexer (Labnet international inc., Edison, NJ) at 35 rpm for 1.5 hours. Headspace samples (100 μL) were injected into a GC (HP5890, MN , equipped with a flame ionization detector (FID)) using a packed column (6 ft ×

1/8 in o.d. 60/80 carbopack B/1% SP-1000, Supelco, Bellefonte, PA). The detection limit was 0.1 mg/L.

For VFAs analysis, 2.7 mL aqueous samples were collected and mixed with 0.3 mL of 0.3-M oxalic acid (Capiro et al. 2008) .Mixtures were then filtered into 1-mL screw-cap vials followed by 1 μL injections into a GC (HP5890, MN) equipped with a FID and a glass column (2 m×2 mm inner diameter) containing 80/120 Carbopack B-DA*/4% Carbowax 20 M (Supelco, Bellefonte, PA). The GC heating program was 175°C for10 min, injection port temperature 200 °C, and FID temperature 200 °C.

Groundwater geochemical parameters analysis

Temperature, pH, ORP, DO and conductivity of groundwater were monitored in Channel 1 by a water quality sonde (YSI 600XLM; YSI, inc. Yellow Springs, Ohio) installed at M2 (Figure 1). The sonde was programmed to take readings at 0:00 am and 12:00 pm daily from April 27th 2009 to June 9th 2010. Sensors were calibrated according to manufacturer protocols.

Soil core sample collection and DNA extraction

Soil core samples in the saturated zone of B1 and B2 were collected for qPCR analysis. The sandy soil core sampling was performed using 1.2 m (1.25 cm) stainless steel piping. The pipe was hammered into the soil using a rubber mallet. When the pipe reached the target depth, the top of the pipe was sealed with rubber septa and the pipe was then extracted by hand. The segments at the depth of 0-25 cm, 25-50 cm and 50-75 cm were taken successively. Each depth was sampled twice to make sure no soil

remained at that depth. Before taking sample from the next depth, the sampling pipe was washed by tap water to remove the residual soil on the pipe wall. Soil sample were dried in weighing boats (Fisher Scientific) at room temperature (24°C) and stored in -80 °C freezer before DNA extraction. Genomic DNA was extracted using a PowerSoil DNA Kit (MOBIO Laboratories, Inc., Carlsbad, CA) in triplicate from the same soil sample (depth of 50-75 cm).

Quantitative real-time PCR (qPCR)

For Taqman PCR reactions, the mixtures contained 12.5μL TaqMan® Environmental Master Mix 2.0 (Applied Biosystems; Foster City, CA), 500 nM each primers, 200 nM probe and 2μL template DNA in a total volume of 25μL. For SYBR Green I PCR reactions, the mixture contained 12.5μL Power SYBR® Green PCR Master Mix (Applied Biosystems; Foster City, CA), 500 nM each primers and 2μL template DNA in a total volume of 25μL. ABI 7500 Sequence Detector (Applied Biosystems) was used to perform qPCR reactions with the following temperature program: 50°C for 2 min, followed by 95°C for 10 min and 40 cycles at 95°C for 15 s, and 30s at annealing temperature for each primers (Table 1), 40s at 72 °C for extension. For SYBR green detection, the melting curve analysis was conducted after thermal cycle complete to make sure no nonspecific PCR products were generated. Primer sequences, reaction chemistries, target gene and standards DNA for calibration curve are summarized in Table 1.

Table 1. primers and probe for qPCR analysis

Target gene	qPCR chemistry	Primer/ probe name	Sequence	Standard	reference
Bacteria 16S rRNA	TaqMan	1055f	5'-ATGGCTGTCGTCAGCT3'	*Escherichia coli*	(Harms et al. 2003)
		1392r	5'- ACGGGCGGTGTGTAC-3'		
		16STaq1115	5'-FAM-CAACGAGCGCAACCC-TAMRA-3'		
Archaea 16S rRNA	TaqMan	ARCH1-1369F	5'-CGGTGAATACGTCCCTGC-3'	*Methanococcus maripaludis*	(Suzuki, Taylor and DeLong 2000, Da Silva and Alvarez 2004)
		ARCH2-1369F	5'-CGGTGAATATGCCCCTGC-3'		
		PROK1541R	5'-AAGGAGGTGATCCTGCCGCA-3'		
		TM1389F	5'-FAM-CTTGTACACACCGCCCGTC0-BHQ-3'		
mcrA	SYBR Green I	MLf	5'- GGTGGTGTMGGATTCACACARTAYGCWACAGC-3'	*Methanocaldoc cus jannaschii*	(Luton et al. 2002)
		MLr	5'- TTCATTGCRTAGTTWGGRTAGTT-3'		
fhs	SYBR Green I	fhs1	5'-GTWTGGGCWAARGGYGGMGAAGG-3'	*Morrella thermoacetia*	(Xu et al. 2009)
		FTHFS-r	5'-GARGAYGGWTTTGAYATYAC-3'		
Bacterio-phage λ	TaqMan		5'-ACGCCACGCGGGATG-3'	Bacteriophage lambda DNA	(Beller et al. 2002)
			5'-AGAGACACGAAACGCCGTTC-3'		
			5'-TET-ACCTGTGGCATTTGTGCTGCCG-TAMRA-3'		

RESULTS AND DISCUSSIONS

Effect of temperature on CH_4 production

Within the channel exposed to the ethanol, dissolved CH_4 in A1 increased from <0.1 mg/L (August 7th, 29.9 °C) to 6.8 mg/L (December 18th, 10.8 °C) and then decreased to less than 0.5 mg/L concomitantly with the lower temperatures in January and February (<10 °C). CH_4 concentration then increased 0.2 mg/L (March 29th) to 12.9 mg/L (June 9th) with the increasing temperatures (from 16.0 °C to 30.0 °C). A similar trend was observed at the B1 sampling well. The maximum CH_4 concentration was 17.9 mg/L (B1, May 29th, 26.9 °C), representing 81% of the solubility limit at the corresponding temperature (Yamamoto, Alcauskas and Crozier 1976). CH_4 was not detected in the control channel (Channel 2) over the 11 month period. The lack of CH4 detection in the control channel may be due to (1) much longer acclimation periods required for BTEX than for ethanol degradation under methanogenic conditions, often requiring years (Da Silva and Alvarez 2004), and (2) the control channel was exposed to a much lower concentration of organic compounds (92 versus 1.3×104 mg/L as total organic carbon) that are potential sources of reducing equivalents for CH4 formation.

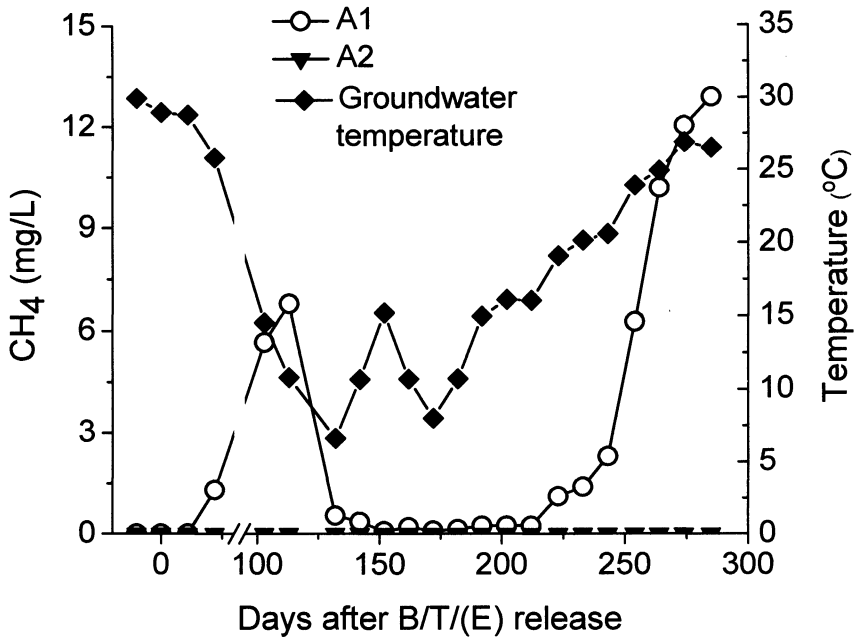

Figure 2. CH4 concentration at sampling well A1 (in Channel 1, exposed to ethanol and B/T) and A2 (in Channel 2, exposed to B/T alone). Sampling wells are depicted in Figure 1. Day 0 corresponds to August 17th, 2009.

A BX 168 portable combustible gas detector (Appendix II) (Henan Hanwei Electronics Co. Ltd, China) (detection limit: 1% of CH_4 lower explosive limit, or 400 ppm_v CH_4) was used to analyze for CH_4 concentrations in the air just above the soil surface of the ethanol-amended channel. No CH_4 was detected, probably due to dilution by air movement as CH_4 reaches the surface as well as to some possible CH_4 biodegradation by methanotrophs in the vadose zone (Bull et al. 2000, King 1997) However, migration of CH_4 from near-source ethanol impacted groundwater and subsequent to accumulation in subsurface enclosed spaces could lead to potential explosion risks where ignitable conditions exist. Thus, further research is needed to delineate conditions that are conducive to CH_4 accumulation to inform the need for

periodic monitoring.

A strong inverse correlation existed between CH_4 production (A1) and water temperature ($p = 0.00009$) (Figure 5 a), which indicates that CH_4 generation from the fuel ethanol blends were significantly influenced by the variation of temperature. The annual average temperature of shallow ground water (10-25m depth) in the U.S. ranges from 4°C in the north central areas to approximately 25°C in southern Florida. The seasonal variation in groundwater temperature is greatest near the surface, amounting 5°C to 10 °C (Heath 1983). Methanogenesis is known to be enhanced at higher temperatures and inhibited by low temperatures (Westermann 1993, Conrad, Schutz and Babbel 1987, Cullimore, Maule and Mansuy 1985) .

Effect of temperature on VFAs production

Acetate concentrations remained below 5 mg/L in the control channel throughout the monitoring period. However, in the channel exposed to the ethanol, acetate concentrations (A1) increased from <1 mg/L (August 7th, 29.9 °C) to 95.7 mg/L (December 8th, 14.6 °C), followed by a concentration decrease to below 40 mg/L in January. (< 10 °C) From February to June, with the subsequent increase in temperature (from 8.0 °C to 30.0 °C), the acetate concentration increased again to 131 mg/L (April 29th). A similar trend was observed at the sampling well B1. The maximum concentration measured was 226 mg/L (B1, May 10th, 23.9 °C). This indicates that acetate production was significantly influenced by temperature variations. Similar to CH_4, a significant inverse correlation was found between acetate production (A1) and temperature (p =

0.007) (Figure 5 b). Apparently, higher temperatures are conducive to faster ethanol biotransformation to VFAs and H_2, which in turn results in higher CH_4 production. Accordingly, higher availability of acetate (which is the main substrate for aceticlastic methanogens) was significantly correlated ($p = 0.027$) to CH_4 concentrations (Figure 6a).

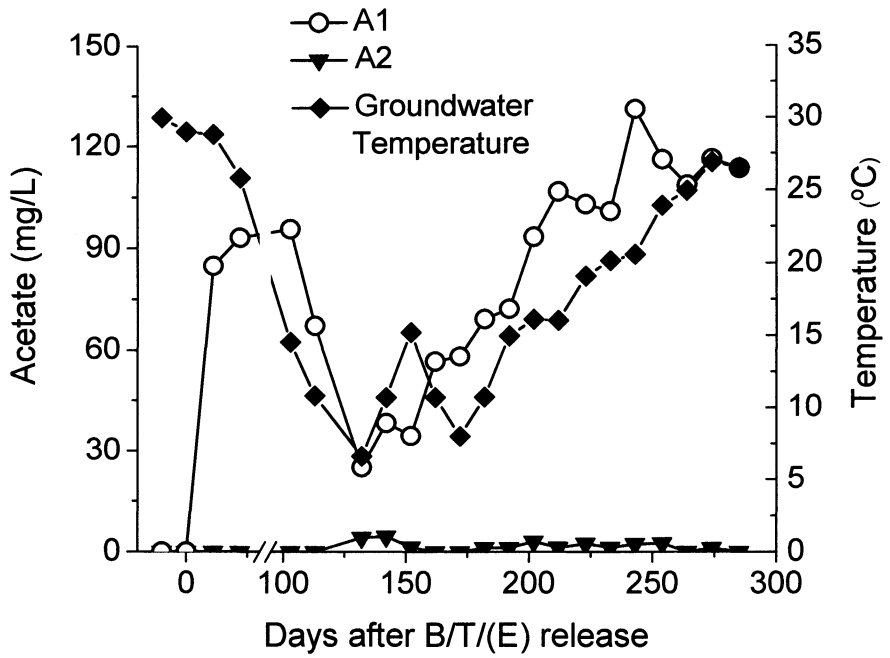

Figure 3. Acetate concentrations at sampling wells A1 (in Channel 1, exposed to ethanol and B/T) and A2 (in Channel 2, exposed to B/T alone). Sampling wells are depicted in Figure 1. Day 0 corresponds to August 17th, 2009.

Unlike acetate, butyrate remained at a relatively low level (< 20 mg/L) from August 7th until late February, and then increased steadily to 280 mg/L (A1, May 29th). The initial lag in butyrate production was expected as butyrate was likely a product of acetate biotransformation. Under anaerobic conditions, ethanol is oxidized to acetate followed by a conversion to acetyl coenzyme A (acetyl-CoA). Two acetyl-CoA can form one

butyryl-coenzyme A (butyryl-CoA), which can then be converted to butyrate (Barker et al. 1945; Gibson 1965). Since acetate is a direct precursor for butyrate formation, its higher abundance is conducive to higher butyrate accumulation, and a significant correlation was found between their concentrations ($p = 0.0012$) (Figure 6b). Accordingly, a significant correlation was also found between butyrate production (A1) and temperature ($p = 0.00000023$) (Figure 5 c).

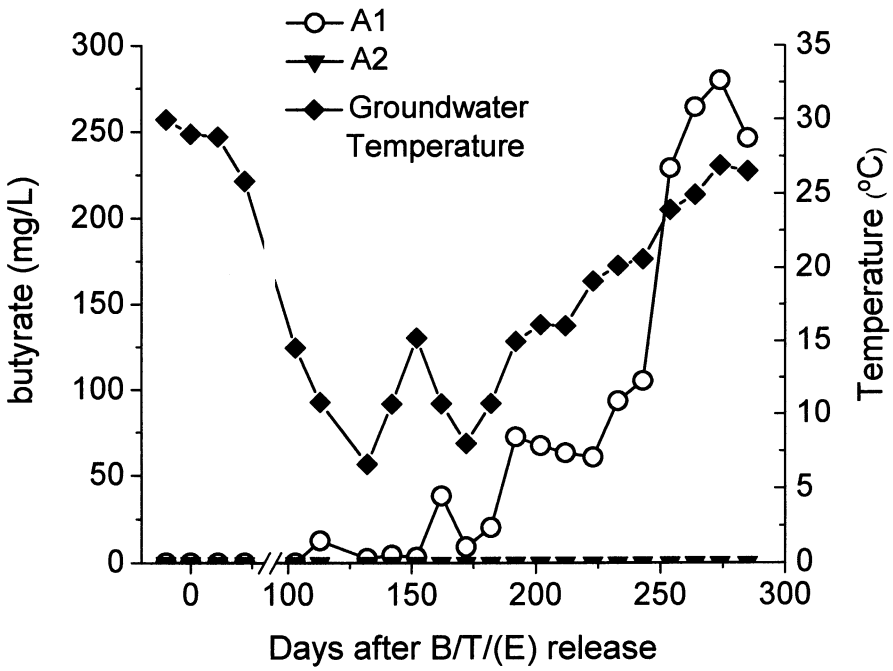

Figure 4. Butyrate concentrations at sampling wells A1 (in Channel 1, exposed to ethanol and B/T) and A2 (in Channel 2, exposed to B/T alone). Sampling wells are depicted in Figure 1. Day 0 corresponds to August 17th, 2009.

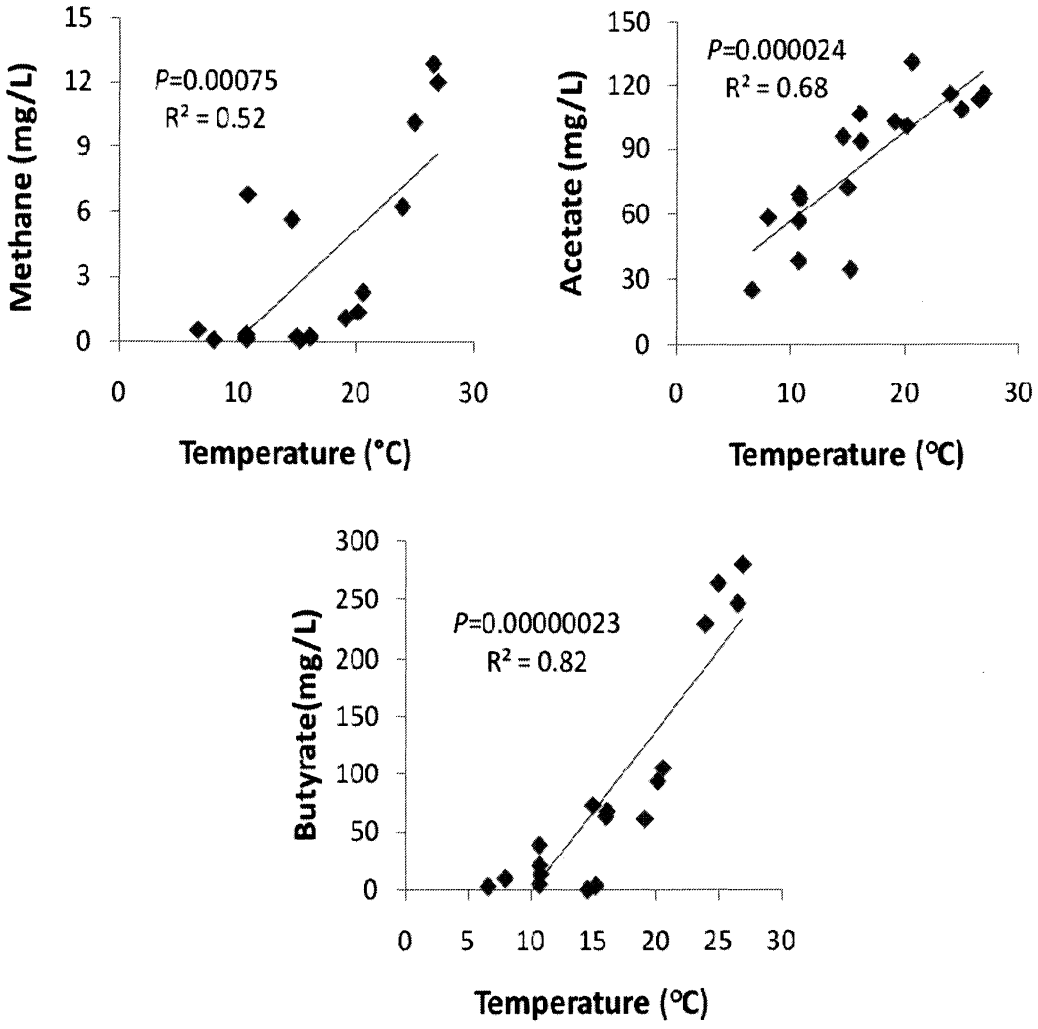

Figure 5. Significant correlations between CH4 (a), acetate (b) and butyrate (b) concentrations (measured at A1) versus groundwater temperature

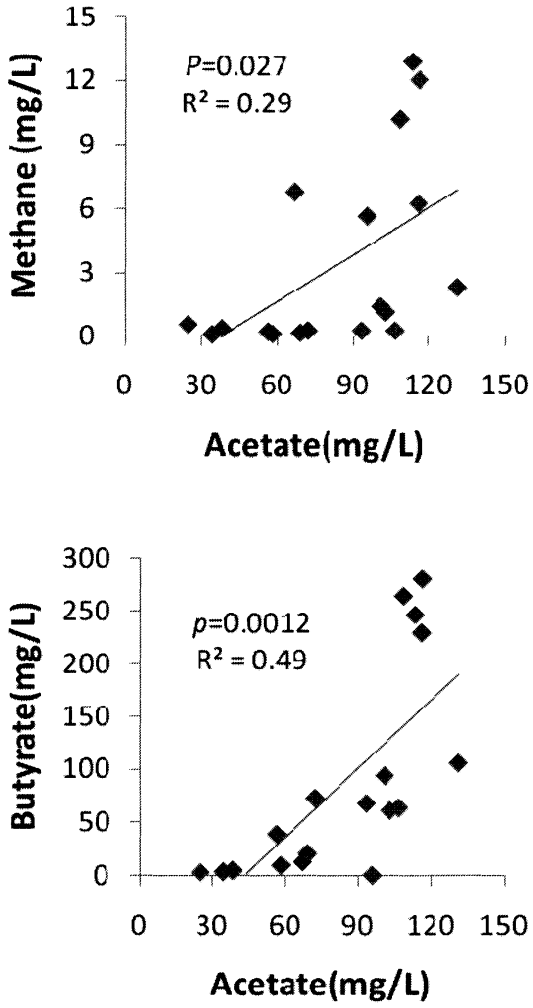

Figure 6. Significant correlations between acetate availability and the formation of (a) CH4 and (b) and butyrate concentrations (measured at A1). Acetate is a precursor to both methane and butyrate formation.

Effects of temperature on ORP, DO and pH

ORP, pH and DO data varied seasonally. The decrease in ORP (from 110 mV to -310 mV), pH (from 7.0 to 5.1) and DO (from 5.3 mg/L to 0 mg/L) following the release of the ethanol blend indicated transition to anaerobic conditions. During January and February, microbial activity was inhibited by low temperatures (< 10 °C), resulting in an

increase in ORP (to 80 mV), DO (to 3.6 mg/L) and pH (to 6.7) thereby shifting the aquifer system from anaerobic to aerobic conditions (Delaune 2005). After March the system reverted back to an anaerobic state indicated by a decrease of ORP (to -400 mV), DO (to < 0.1 mg/L) and pH (to 4.6) thereby corroborating the relationship in ORP, pH, and DO with temperature.

VFAs Odor Generation

The standard odor criterion (Secondary Maximum Contaminant Level or SMCL) for the US EPA National Secondary Drinking Water Regulations is the threshold odor number (TON) = 3. The TON is defined as the greatest dilution of sample with odor-free water yielding a definitely perceptible odor (Greenberg, Clesceri and Eaton 1992). We determined the TON for each VFAs species according to equation 1:

$$\text{Threshold odor number} = \frac{\text{Odorant concentration } (C_{gas})}{\text{Odor threshold value for that odorant}} \quad (1)$$

The "odor threshold value" is the lowest concentration of a specific odorant detectable by human olfaction. The "odorant concentration" is the gas phase concentration (C_{gas}) of a specific odorant (e.g., VFAs), which can be calculated based on measured aqueous concentration (C_{aq}). Note that C_{aq} is the total concentration comprising both the weak acid (the protonated form susceptible to volatilization) and its conjugated base (which is charged and not susceptible to volatilization). The concentration of the protonated form that can undergo volatilization (and thus generate odor), C_{HA}, can be calculated based on the measured C_{aq}, the pH of the solution, and the corresponding

acid/base equilibrium constant (Ka) and molecular weight (MW) according to equation 2:

$$C_{HA}(mol/L) = \frac{C_{aq}(mg/L)\times 10^{-3}(g/mg)}{MW(g/mol)\times(1+K_a/10^{-pH})} \quad (2)$$

C_{gas} can be calculated using Henry's law (equation 3), where K_H is Henry's law constant:

$$C_{gas}(ppm_v) = \frac{C_{HA}(mol/L)\times 10^3(L/m^3)\times K_H(atm\cdot m^3/mol)}{1\,atm}\times 10^6 \quad (3)$$

Two representative samples of different seasons (A1, Jan 8th and A1, May 29th) were chosen to assess the seasonal variation of odor generation. The groundwater temperature and pH for these two samples were 6.6 °C, pH 6.6 for (A1, Jan 8th) and 26.9 °C, pH 4.6 for (A1, May 29th). Table 2 summarizes the calculated C_{gas} values, and Table 3 depicts the odor threshold value for each VFAs and the TON values for each sample.

Table 2. Calculated gas phase concentrations of VFAs

VFAs	Measured C_{aq} (mg/L)	pKa	C_{HA} (mol/L)	Henry's law constant (atm m^3/mol) [b]	C_{gas} (ppm_v)
Summer (sampled at A1; May 29th;26.9 °C)					
Acetic acid	116	4.75[a]	1.02×10^{-3}	1.08×10^{-7}	1.10×10^{-1}
Propionic acid	7	4.87[a]	5.64×10^{-5}	4.42×10^{-7}	2.29×10^{-2}
Butyric acid	280	4.85[a]	1.86×10^{-3}	5.62×10^{-7}	1.04
Winter (sampled at A1; Jan 8th; 6.6 °C)					
Acetic acid	25	4.75[a]	5.80×10^{-6}	4.58×10^{-8}	2.66×10^{-4}
Propionic acid	4	4.87[a]	9.87×10^{-7}	2.17×10^{-7}	2.15×10^{-4}
Butyric acid	3	4.85[a]	5.95×10^{-7}	3.23×10^{-7}	1.92×10^{-4}

Source: [a] (Schwarzenbach, Gschwend and Imboden 2002)

[b] Henry's constants were obtained from (Howard 1990) for acetic acid, and from (Howard 1997) for propionic and butyric acids. These constants were corrected for the corresponding temperature using the Van't Hoff equation, using standard enthalpy values from Haynes (2010)

Table 3. VFAs threshold odor number

VFAs	Odor threshold value (ppm_v)	C_{gas} (ppm_v)	Threshold odor number (TON)
	Summer (sampled at A1; May 29th;26.9 °C)		
Acetic acid	1[(a)]	1.10×10^{-1}	0.1
Propionic acid	0.0057[(b)]	2.29×10^{-2}	4.4
Butyric acid	0.001[(a)]	1.04	1045
	Winter (sampled at A1; Jan 8th; 6.6 °C)		
Acetic acid	1[(a)]	2.66×10^{-4}	< 0.1
Propionic acid	0.0057[(b)]	2.15×10^{-4}	< 0.1
Butyric acid	0.001[(a)]	1.92×10^{-4}	0.2

Source: [(a)](Cheremisinoff 1999) [(b)] (Nagata 2003)

For simplicity, we assumed that only acetic acid, propionic acid and butyric acid contribute to the odor in the groundwater sample. The threshold odor number of the summer sample (A1, May 29th) (1049.5 TON) was much larger than the SMCL, and butyric acid was the major contributor to odor generation. The threshold odor number of the winter sample (A1, Jan 8th) (<0.4 TON), however, was lower than the SMCL. As discussed previously, lower temperatures decreased microbial activities (including transformation of ethanol into VFAs) which mitigated odor generation. Overall, the

results indicate that near a source, ethanol=blended releases to groundwater can generate odor problems that compromise water quality, but the level of impact would likely vary seasonally.

Impact of ethanol blend on the indigenous microbial community

Soil samples taken at B1 before the release started (baseline sample, August 9th 2009) and after 10 months of continuous release (June 21th 2010) were chosen to access the impact of long term release of ethanol blend to the indigenous microbial community. *Bacteria* 16S rRNA gene copy numbers taken on June 21th 2010 (9.3×10^7 copy/g dry soil) were 14 times higher than those in baseline sample (6.5×10^6 copy/g dry soil), while *Archaea* 16S rRNA gene copy numbers taken on June 21th (3.6×10^7 copy/g dry soil) were 110 times than those in baseline sample (3.3×10^5 copy/g dry soil). As an easily degraded compound, ethanol represents a good carbon and energy source that can stimulate both *Bacteria* and *Archaea* growth. Thus, higher biomass would be expected at the downstream of ethanol blends source.

Compared with the baseline sample, acetogenic and methanogenic groups at B1 proliferated after 10 months of continuous ethanol blend release (Figure 7). Methanogenesis functional gene (*mcrA*) copy numbers in the sample of June 21th 2010 was 4.2×10^6 copy/g dry soil, but was not detected in the baseline sample (Detection limit: 5000 gene copy/ g dry soil). Acetogenesis functional gene (*fhs*) numbers in the sample of June 21th 2010 (2.6×10^8 copy/g dry soil) were 87 times higher than those in the baseline sample (3×10^4 copy/g dry soil). The abundance of fermentation functional genes at B1

are consistent with the relatively high methane and acetate concentrations measured there and reflect the strong fermentation activity stimulated by the ethanol. Figure 8 shows the relative abundance of methanogenesis (*mcrA*) and acetate fermentation functional gene (*fhs*) copy numbers relative to total 16S rRNA gene copy numbers (All the known methane generation microorganism belong to *Archaea* and all the known acetate fermentation microorganism belong to *Bacteria*). The ratio of *mcrA* copy number to *Archaea* 16S rRNA gene copy number in the sample of June 21th 2010 was 11.6% ± 5.5% while in the baseline sample the *mcrA* gene was undetected (Detection limit=5000 copy/ g dry soil). The ratio of *fhs* copy number to *Bacteria* 16S rRNA gene copy number in the sample of June 21th 2010 was 2.8% ±1.4% while the ratio in the baseline sample was 0.2±0.1%. The ethanol in aquifer posed a selective pressure to the indigenous microbial community. The microorganism that is able to utilize ethanol (e.g. fermentation groups) would win the competition with other species and their population would proliferate. Thus, ethanol can selectively enrich the fermentation groups and change the composition of microbial communities.

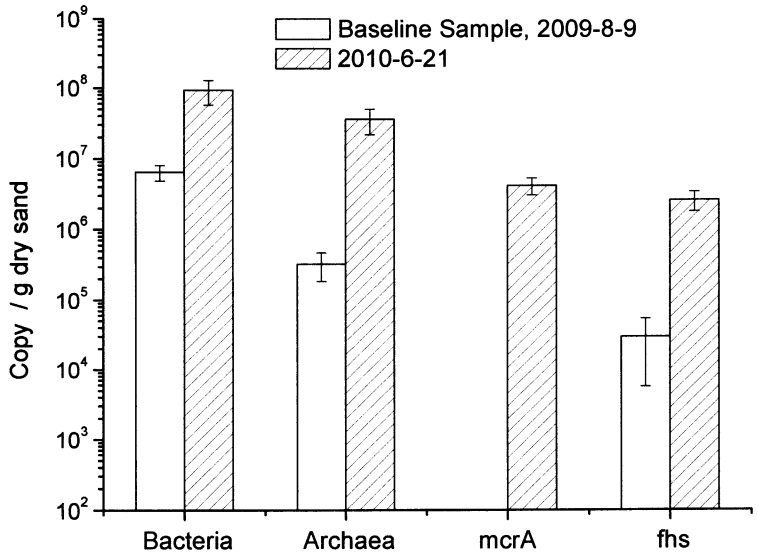

Figure 7. Comparison of microbial population in baseline sample (August 9th 2009) and the sample taken following 10 months of continuous ethanol release (June 21th 2010). *mcrA* gene in baseline sample was under detection limit (5000 copy /g dry soil)

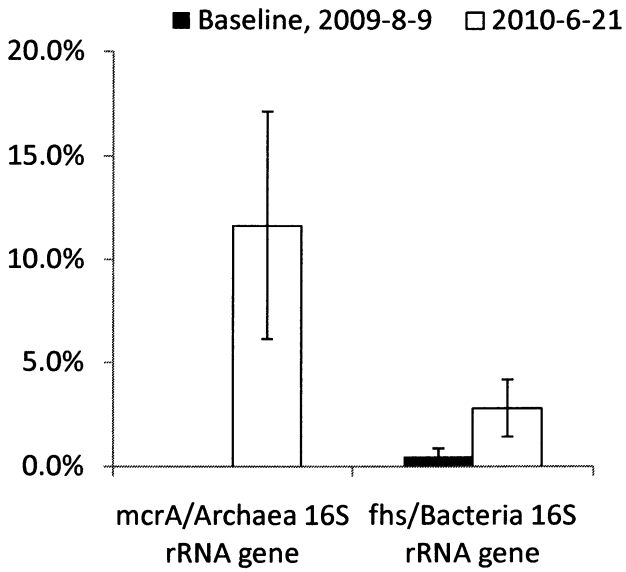

Figure 8. Relative abundance of methanogenesis and acetate fermentation functional gene copy numbers relative to total 16S rRNA gene copy numbers in the baseline sample (August 9th 2009) and taken after 10 months of continuous ethanol release (June 21th 2010). *mcrA* gene in the baseline sample was under detection limit (5000 copy /g dry soil)

CONCLUSIONS

As an easily degraded organic compound, ethanol represents a good carbon and energy source that can stimulate the growth of *Bacteria* and *Archaea*. The introduction of ethanol blend into the pilot aquifer system changed the composition of indigenous microbial community, enriching the genotypes related with the fermentative degradation of ethanol. Temperatures significantly affected the biodegradation of ethanol blends. A direct, significant ($p < 0.05$) correlation was observed between groundwater temperature and CH_4/VFAs accumulations. The accumulations of VFAs in the groundwater can cause serious malodor problems. The main contributor to water odor was butyric acid, which accumulated at levels that exceeded the SMCL stipulated by National Secondary Drinking Water Regulations. The production of methane up to an aqueous concentration of 17.9 mg/L did not result in detectable concentrations on the ground surface (40 cm above the water table)..

ENGINEERING SIGNIFICANCE AND RECOMMENDATIONS

The results in this thesis show that the accumulation of VFAs in groundwater may cause previously overlooked aesthetic impacts. Seasonal variations in groundwater temperatures can affect the VFAs generation with warmer summer temperatures resulting in stronger odor. The presence of odorants in drinking water affects the public's perception of the drinking water safety and malodor is one of the main reasons for consumers to complaint about their drinking water quality. Therefore, seasonal variations

of odor generation and methane accumulation in subsurface environments (or subsurface confined spaces) should be considered at the sites contaminated with fuel ethanol blends.

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Appendix I

Inlet Water Characteristics

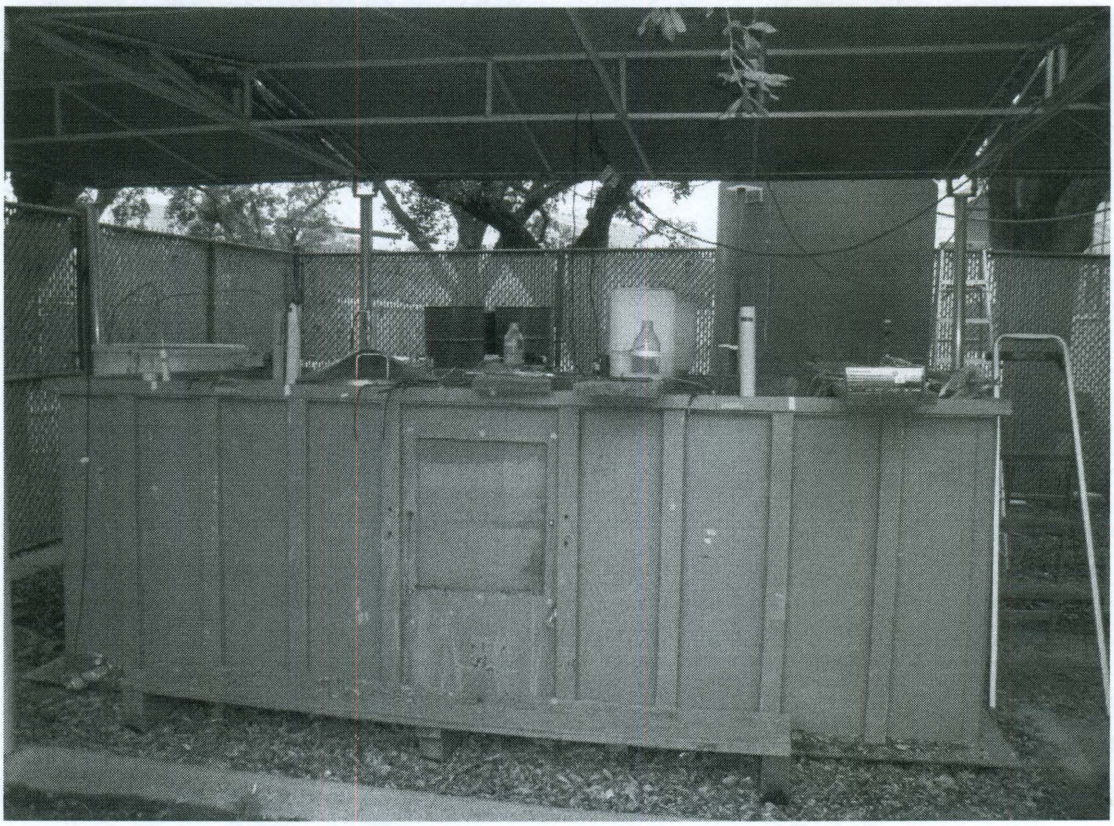
Inlet water characteristics

Parameter	Value (± stdev, n = 158)
Flow rate (L/day)	170 ±40 L/day (each channel)
pH	7.2 ± 0.4
Dissolved oxygen (mg/L)	7.1
Ionic strength (mM)	6-12

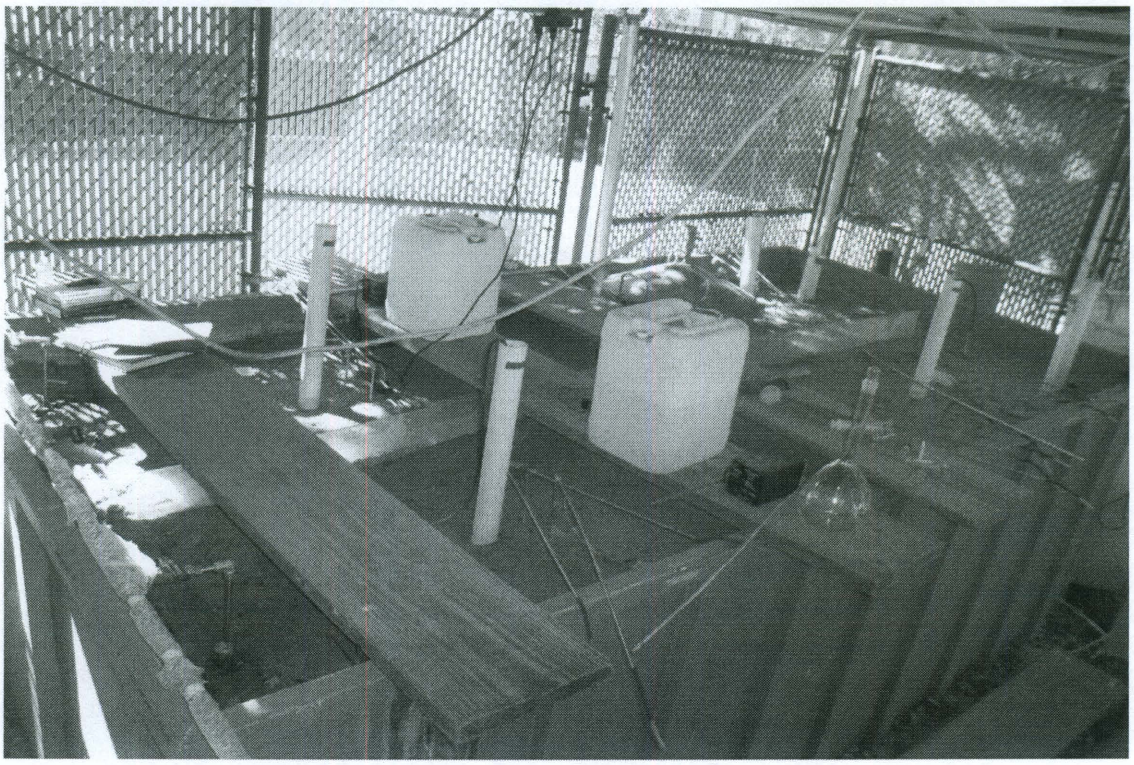
Appendix II

Aquifer Tank Pictures

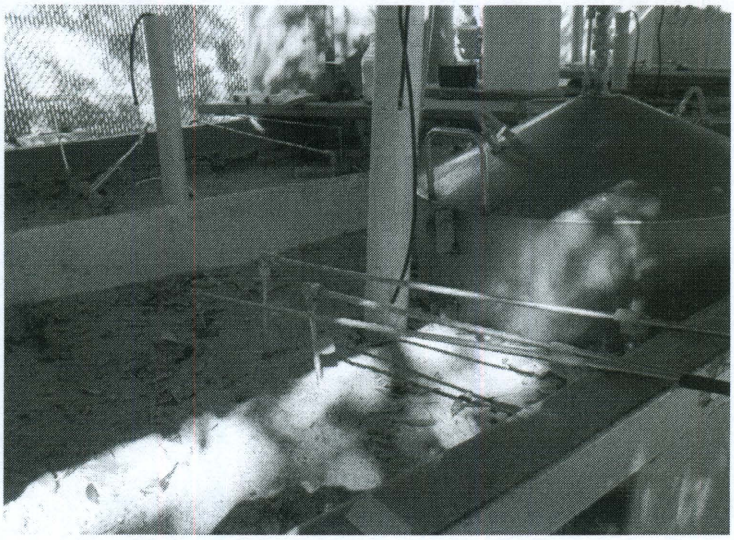
Side-glance



Top View

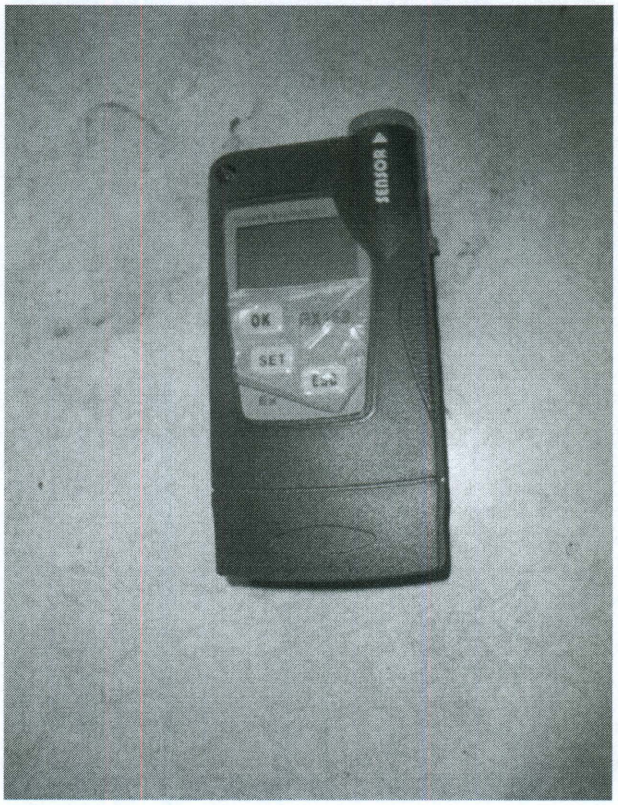


Groundwater sampling ports



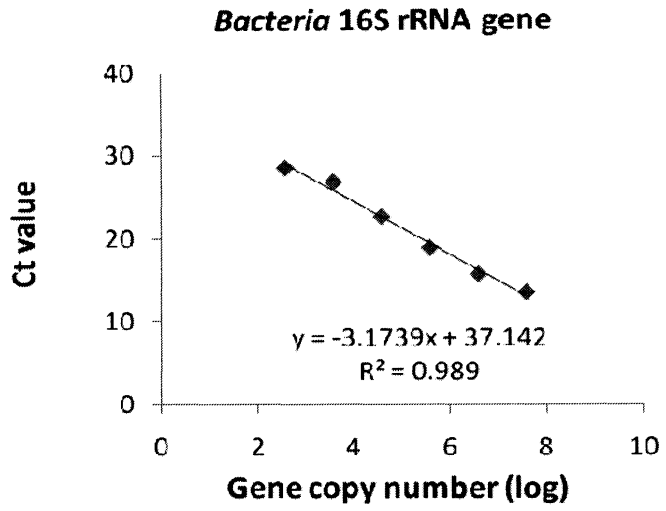
Ethanol blend injection ports

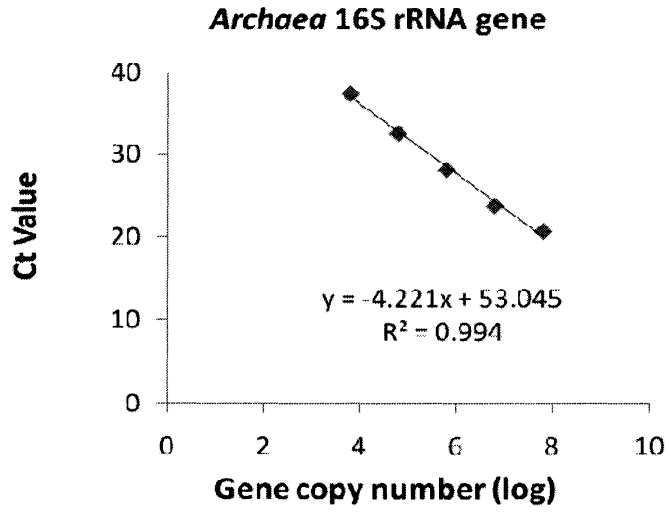
BX 168 portable combustible gas detector

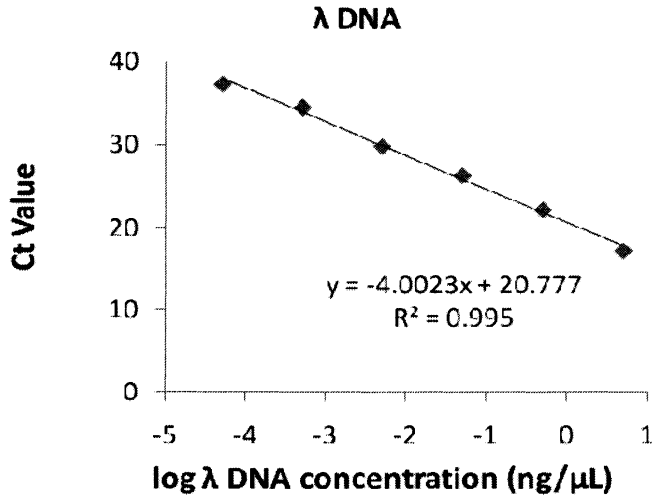


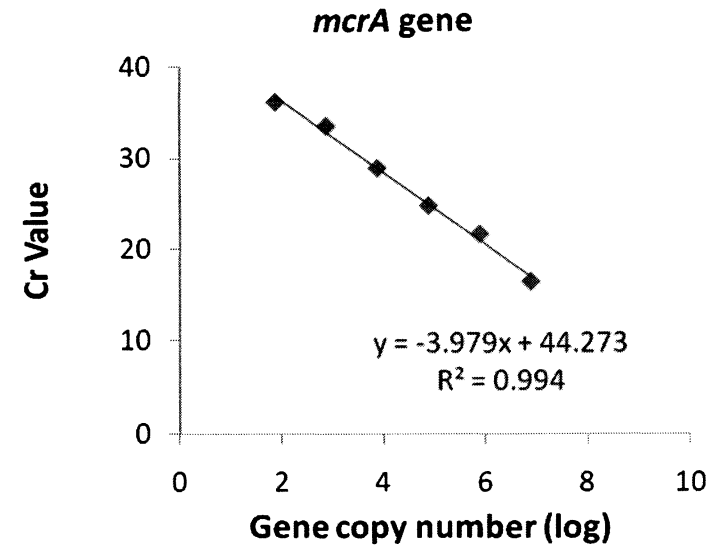
Appendix III

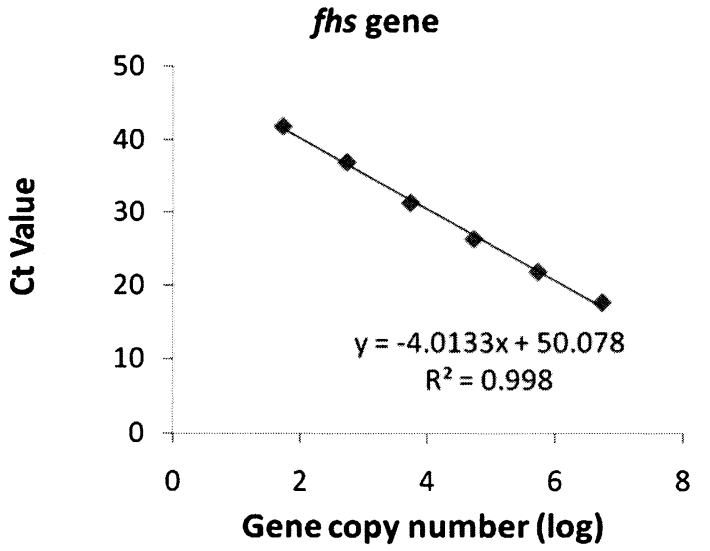
Calibration Curve for Quantitative Real-Time PCR


Bacteria 16S rRNA gene
Ct value
40
30
20
10
0
y = -3.1739x + 37.142
R² = 0.989
0
2
4
6
8
10
Gene copy number (log)


Archaea 16S rRNA gene
Ct Value
40
30
20
10
0
y = -4.221x + 53.045
R² = 0.994
0
2
4
6
8
10
Gene copy number (log)

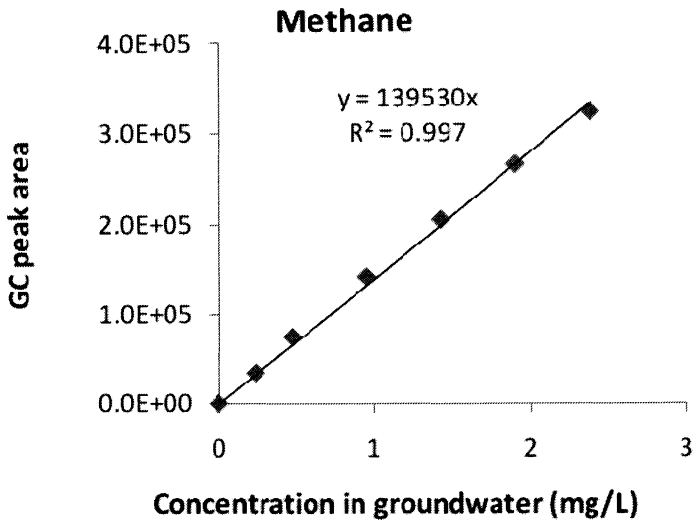

λ DNA
Ct Value
40
30
20
10
0
y = -4.0023x + 20.777
R² = 0.995
-5
-4
-3
-2
-1
0
1
log λ DNA concentration (ng/μL)

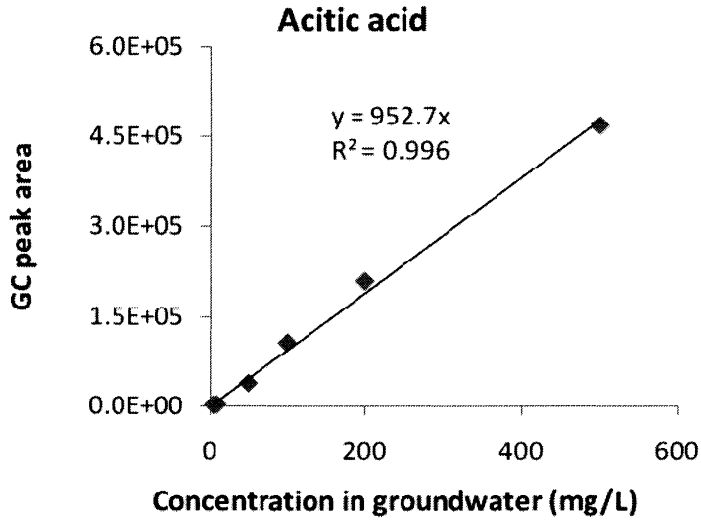

mcrA gene
Cr Value
40
30
20
10
0
y = -3.979x + 44.273
R² = 0.994
0
2
4
6
8
10
Gene copy number (log)

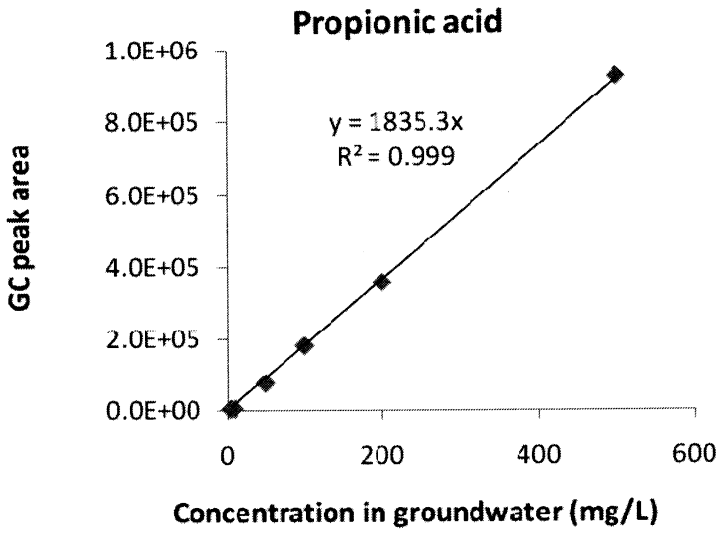

fhs gene
Ct Value
50
40
30
20
10
0
y = -4.0133x + 50.078
R² = 0.998
0
2
4
6
8
Gene copy number (log)

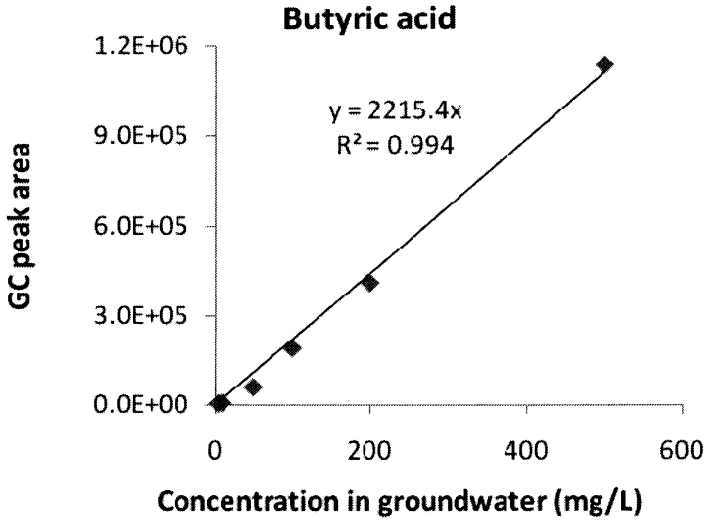
Appendix IV

Calibration Curve for Methane and VFAs analysis


Methane
y = 139530x
R² = 0.997
GC peak area
4.0E+05
3.0E+05
2.0E+05
1.0E+05
0.0E+00
0
1
2
3
Concentration in groundwater (mg/L)


Acitic acid
y = 952.7x
R² = 0.996
GC peak area
6.0E+05
4.5E+05
3.0E+05
1.5E+05
0.0E+00
0
200
400
600
Concentration in groundwater (mg/L)


Propionic acid
1.0E+06
8.0E+05
6.0E+05
4.0E+05
2.0E+05
0.0E+00
y = 1835.3x
R² = 0.999
GC peak area
0
200
400
600
Concentration in groundwater (mg/L)


Butyric acid
1.2E+06
9.0E+05
6.0E+05
3.0E+05
0.0E+00
y = 2215.4x
R² = 0.994
GC peak area
0
200
400
600
Concentration in groundwater (mg/L)